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(54) Synthesis of modified diaminopurines and their incorporation into oligomers

(57) Protected diaminopurines can be used in the synthesis of nucleic acid binding compounds containing diaminepurine in place of other nucleobases. A method for the synthesis of protected diaminopurines is disclosed which is convenient for incorporating modified diaminopurines into monomers or into oligomers for the preparation of nucleic acid binding compounds.

EP 1 085 020 A1

Description

[0001] Subject of the present invention are methods for the preparation of modified diaminopurines, preferably protected diaminopurines, methods for incorporating said modified diaminopurines into monomers, methods for the synthesis of oligomers using these monomers as well as nucleic acid binding compounds containing said diaminopurines.

[0002] Non-natural nucleic acid binding compounds have been found to be valuable mimics for nucleic acids, especially mimicking or even improving the nucleic acid binding features of DNA and RNA. Peptide Nucleic Acids (PNA) have been shown to form thermally very stable duplexes and triplexes with complementary RNA and DNA oligonucleotides (Egholm et al. Nature, 1993, 365, 566- 568). Further substitution of adenine (A) by 2,6-diaminopurine (D) has been reported to raise the melting point (T_m) for DNA/DNA and RNA/DNA hybrids (Hoheisel et al. FEBS 1990, 274, 103; Lamm et al. Nucleic Acids Research 1991, 19, 12 3193). This effect on the T_m is depending upon the nucleo base sequence of the DNA oligonucleotide. Thus, the stabilization compared to duplexes formed from the DNA and an oligonucleotide containing only A is most pronounced in mixed sequences. There may even be a destabilizating effect in poly adenine sequences (Cheong et al., Nucleic Acids Research, 1988, 16,11, 5115).

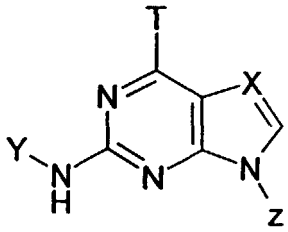
[0003] Diaminopurine monomers have been prepared usually via a precursor molecule in which the 9-carbon atom (C9) is capped and thereby protected by an electron donating group, such as a substituted alkyl group. For the coupling of the precursor to an amino group of a monomeric backbone unit, the amino group at the C6 of the base moiety had to be protected. This was earlier made by reacting the precursor with benzyloxycarbonyl imidazolide. However, this reaction was not selectively taking place at the C6 atom. Such preparation is disclosed for example in Haaima et al., Nucl. Acids Res. 1997, 25, 4639-4643, WO 92/20702 and US-5,705,347.

[0004] It is one object of the present invention to provide a convenient synthesis of diaminopurines modified at the exocyclic amino groups in position C6 and preferably also in position C2 of the purine ring structure.

[0005] It is a further object of the invention to provide a method for incorporating modified diaminopurines into monomers for the preparation of nucleic acid binding compounds

[0006] It is a further object of the present invention to provide a synthesis of diaminopurine containing nucleic acid binding compounds with less occurrence of side products.

[0007] Subject of the invention is a method for the synthesis of compounds of the general formula I



(I)

wherein

T is NV^1V^2

wherein

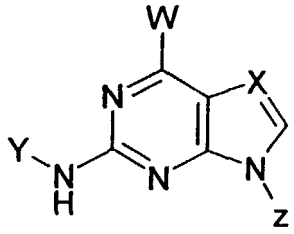
V^1 and V^2 are linked together other than via the N shown in the definition by a chain of at least 5 atoms, or is NHV, wherein V is selected from the group consisting of hydrogen, alkyl, a solid phase, a protecting group, and a reporter group

X is CH or N,

Y is an electron withdrawing protecting group, preferably a group belonging to the group of acyl groups, unsubstituted or substituted by, and

Z is a not electron withdrawing group, preferably is an electron donating group, most preferably selected from the group consisting of hydrogen (H) alkyl, unsubstituted or sustituted by a substituent selected from the group of SO_3H, PO_4H, COOH, CN and derivatives, like esters, thioesters or amides, thereof, said derivatives including, but being not restricted to an unprotected or protected nucleic acid binding compound, a partially or fully protected monomeric backbone unit of a nucleic acid binding compound,

characterized in that a compound of general formula II



wherein
the definitions of X, Y and Z are chosen from the possible definitions of X, Y and Z in formula I and

W is an electron withdrawing group, preferably selected from the group consisting of NO_2, halogen, Tos and Mes,

is reacted with a compound of general formula III,

T-H (III)

wherein

T is defined as in formula I,

under conditions favoring the elimination of a compound of the formula WH.

[0008] Any substituent in Z may or may not be altered during the reaction to yield another substituent within the possible definitions of said substituents in Z. One example for such reaction including altering substituents is the reaction wherein under said conditions group R^3 (as defined later) is changed from an alkyl group to hydrogen or a alkali metal ion or an ammonium ion. However, such alteration can also be performed in a separate step, using conventional ester hydrolysis.

[0009] Tos means the tosyl group and Mes means the mesyl group. Preferred halogens are iodine (I), Br and Cl, most preferred Cl.

[0010] Preferably, in formula I, V is the group



wherein

R^1 and R^2 chosen independently from the group consisting of hydrogen, alkyl, aryl or allyl or R^1 and R^2 at one C at a defined position are together oxygen
m is an integer of from 0 to 10, preferably from 1 to 3, and
Ar is an aromatic group of less than 15 carbon atoms, preferably of from 6 to 10 carbon atoms.

[0011] Preferred definition of T comprising linked substituents V^1 and V^2 are N-heterocyclic groups derived from secondary amines, like the piperidyl group or the morpholino group. However, such definition will also include compounds comprising aromatic moieties or other double bond containing moieties, as long as at least one secondary amino group is contained. The chain of atoms will be preferably from 5 to 8, most preferably 6, atoms long. Preferred atoms in the chain are carbon atoms and nitrogen and oxygen atoms. Within the definitions of general formula III, V is preferably an amino-protecting group. A preferred definition of V is the benzyloxycarbonyl group. Preferred amino protecting groups V are groups that can be selectively removed after the synthesis from the exocyclic amino group.

[0012] Alkyl groups in the definitins of all formulae, if not otherweise mentioned, preferably contain from 1 to 6, most preferably from 1 to 3, carbon atoms. Acyl groups preferably contain from 2 to 21 carbon atoms, including acyl groups from alkanes including from 1 to 6 carbon atoms unsubstituted or substituted by one or more aromatic groups of from 6 to 14 carbon atoms, and from aromatic moieties, like arenes including from 6 to 14 carbon atoms. Preferred alkylacyl

groups are isobuturyl, t-buturyl, isopropy and acetyl and preferred aromatic acyl is benzoyl. An aromatic group or aryl group is a group containing at least one aromatic ring, unsubstituted or substituted by one or more, but usually less than 3, substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, nitro, halogen or carboxyl. Preferred aromatic groups are the phenyl and the naphthyl group.

[0013] A protecting group of a particular group is a group which prohibits the particular group to participate in any undesired chemical reactions. Preferably, the protecting group can be removed from the particular group thus setting the particular group free. Protecting groups, for example for amino groups, hydroxyl groups and carboxyl groups are generally wellknown to the man skilled in the art, for example from text books on oligonucleotide or peptide synthesis. Protecting groups for amino groups include acyl groups or carbonyloxyalkyl, for hydroxyl groups include acyl groups and for carboxyl groups include alkyl groups. The electron withdrawing and electron donating capability is compared to hydrogen.

[0014] An allyl group is a group containing a carbon-carbon-double bond. Preferred allyl groups contain from 2 to 4 carbon atoms.

[0015] An aralkyl group is an alkyl group substituted by an aryl group. Preferred aralkyl is the benzyl group.

[0016] Preferred definitions of T consist of NH, NCH_3 and piperidyl.

[0017] The preferred definition of X is N.

[0018] The preferred definition for Y is iso-butyryl (C_3H_7CO).

[0019] All these compounds of general formula I can according to the present invention be prepared by substituting group W by the amino group of the compound of formula III. This reaction can be considered as a nucleophilic substitution reaction driven by the exit of a compound WH. Aromatic nucleophilic substitutions at heterocycles are principally known to a man skilled in the art, but have never been applied to diaminopurines. The present invention has found that this type of reaction is of considerable advantage over other methods for preparing such protected diaminopurines, for example, the direct protection of diaminopurine using reactive protecting reagents for the free primary exocyclic amino groups.

[0020] Depending upon the choise of the definition of Z, the compounds of formula I may have very different uses.

[0021] The compounds of formula I, wherein

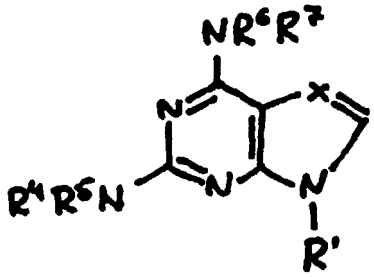
Z is the group R'

$$-(CR^1R^2)_n\text{-}CO\text{-}OR^3 \quad (R')$$

wherein

R^1 and R^2 for each C at different n are chosen independently from the group consisting of hydrogen, alkyl, aryl or allyl or R^1 and R^2 at one C at a defined position are together oxygen and
R^3 is hydrogen or a carboxylic acid protecting group and n is an integer of from 1 to 5 are useful as precursors for the synthesis of diaminopurine containing monomers for the synthesis of oligomers.

[0022] A further subject of the invention is a compound of general formula IV



wherein

R' is the electron donating group $(CR^1R^2)_n$ $COOR^3$
R^4, R^5, R^6, R^7 are independently selected from the group consisting of H and R^8 wherein preferably at least one of R^6 or R^7 is not H,
R^3 is -H, alkyl (unsubstituted or unsubstituted as above), a monomeric backbone unit or a totally or partially pro-

tected or unprotected nucleic acid binding compound,

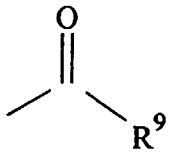
R^8 is alkyl or a protecting group, preferably selected from the group consisting of acyl, aralkyl, carbonyloxyalkyl and carbonyloxyaralkyl,

n is an integer of between 1 and 5,

X is N or CH.

[0023] Preferred compounds of formula IV are those, wherein at least one of R^4 or R^5 is not H or alkyl.

[0024] More preferred are compounds of formula IV, wherein one of R^6 or R^7 and one of R^4 or R^5 is the group



wherein

R^9 is selected from the group of substituted or unsubstituted alkyl, aryl, aralkyl and OR^{10}, wherein R^{10} is H, alkyl, allyl, a reporter group or aryl.

[0025] The compounds of formula I, wherein Z is H, are useful compounds for the preparation of compounds of formula I, wherein Z is the group R' as defined above. The synthesis of the latter compound will include reacting a compound as defined before, wherein Z is H with a compound of the formula V

$$R'—U \qquad (V)$$

wherein U is a good leaving group, like halogen, tos or mes, under conditions favoring nucleophilic substitution. Preferred compounds of formula V are α-halogen substituted compounds, like α-halogen alkyl carboxylic acid esters.

[0026] Thus these compounds and their synthesis are a further subject of the invention.

[0027] Nucleic acid binding compounds are defined according to the present invention by their capability of binding to a nucleic acid via hydrogen bonds of basepairing between complementary bases of both the nucleic acid and the nucleic acid binding compound. The binding can be either in duplex form (for example containing one strand of a nucleic acid and one strand of the nucleic acid binding compound) or in triplex form (for example containing one strand of the nucleic acid and two strands of the nucleic acid binding compound). Preferred nucleic acid binding compounds are not naturally occurring. Examples are disclosed in

[0028] Jones, A.S., Int. J. Biol. Macromol. 1979, 1, 194; De Koning, H., Pandit, U.K., Rec. Trav. Chim., 1971, 91, 1069; Takemoto, K., Inaki, Y., Polym. Mat. Sci. Eng., 1988, 58, 250; Weller, D.D., Daly, D.T., Olsen, W.K., Summerton, J.E., J. Org. Chem., 1991, 56, 6000; Garner, P., Yoo, J.U., Tett. Lett., 1993, 34, 1275; EP-A-0 672 700; EP-0 646 595 or WO 86/05518.

[0029] Most preferred nucleic acid binding compounds are disclosed in WO 92/20702. To this most preferred group of nucleic acid binding compounds further any derivatives and specific embodiments are counted. In WO 94/25477 novel peptide nucleic acids are described, WO 96/02558 is directed to linked PNAs and WO 96/20212 is directed to PNAs incorporating a chiral backbone. WO 95/14708 is directed to compounds having chelating moieties bound to the nucleic acid binding compounds and WO 95/16202 is directed to nucleic acid binding compounds having an enzyme substrate attached thereto, WO 95/08556 discloses chimeric molecules of nucleic acids and nucleic acid binding compounds. The synthesis of such compounds is described in detail therein. Therefore, reference is made with respect to the chemical structure especially the backbone, to these documents. These nucleic acid binding compounds are usually prepared using a strategy requiring protection of any functional groups within the compound during extension of the backbone, such that any functional groups like primary amino groups or hydroxy groups not intended to be extended in the backbone extension are protected by appropriate protecting groups which can be removed in a final step to set free the functional groups in the nucleic acid binding compound. Such functional groups include preferentially any amino groups at nucleobase moieties, like the exocyclic amino groups of cytosine, adenine and guanine. Such protecting groups are known from WO 92/20702, WO 93/12129 and WO 95/17403. The most preferred protecting group hitherto known is the acyl group and the Z group (benzyloxycarbonyl). For any amino groups at the backbone, the Boc group (tert-butyloxycarbonyl is well established.

[0030] Within peptide nucleic acid, such groups include a carboxyl group and an amine group, preferentially pri-

mary amine group. As disclosed in WO 92/20702, the amine group is preferentially protected by the Boc group, but other protecting groups may be the Mmt or the Fmoc groups. Carboxyl groups may be protected using the benzyl group (Bn) forming a carboxylic acid ester. The term "partially protected" means that not all functional groups are protected, but at least one of them. While the fully protected monomeric backbone units may be produced preferentially in the synthesis of such monomeric backbone unit, partially protected backbone units are the preferred starting compounds for attaching the monomeric backbone unit to solid phase, a further monomeric backbone unit or a nucleic acid binding compound to be extended. Preferentially, in case of peptide nucleic acid, such a partially protected monomeric backbone unit contains protected primary amino group as well as a free carboxylic acid group.

[0031] The compounds of formula I, wherein Z is a partially or fully protected monomeric backbone unit of a nucleic acid binding compound are useful for the preparation of such nucleic acid binding compounds by oligomerisation of monomeric backbone units, either different or identical. In the definition of such compounds, group Z is meant to include any linkers, which preferably are situated between the position of the attachment of Z to the 5-ring heterocycle in formula I and the first atom within a linear chain of monomeric backbone units. Preferably, such linker will be derived from any compounds having a length of from 1 to 6 atoms, carbon atoms or/and oxygen or nitrogen, preferably derived from the group R' as defined above. The attachment of the linker to the monomeric backbone unit is preferably via a hetero atom, preferably via a nitrogen atom. Most preferred, the linkage between the linker and the backbone unit will be a peptide bond (CO-NH).

[0032] Such compounds can be prepared analogously to monomers, wherein the definitions of the substituents at the heteroaromatic moiety do not apply as in formula I. For example, in the synthesis of monomers of the synthesis of PNA, a compound of formula I wherein Z is defined to be R', wherein R^3 is an alkyl or aralkyl group (preferably aralkyl), is reacted with a monomeric backbone unit containing a not protected amino group under conditions favoring the elimination of an alkyl or aralkyl alcohol, thus forming an amide.

[0033] A further subject of the invention is therefore a method for the preparation of compounds of formula I, wherein Z is a monomeric backbone unit of a nucleic acid binding compound, comprising reacting a compound of formula I, wherein Z is R', wherein R^3 is an alkyl or aralkyl group with a monomeric backbone unit containing a not protected amino group under conditions favoring the elimination of an alkyl or aralkyl alcohol. However, surprisingly, such monomers can be synthesised in high yield and with reduced loss of protecting groups by reacting a compound of formula II, wherein Z is a partially or fully protected monomeric backbone unit of a nucleic acid binding compound, with a compound of formula III as outlined above. However, in order to avoid the preparation of guanidine side products, alkaline conditions should be avoided. The useful synthesis has been proven for ammonia and other primary amines and it is expected, based on these experiments, that it will work also for other nucleophilic compounds.

[0034] In an overall view of monomer synthesis, the synthesis of monomers via compounds of formula II has the principal advantage that the route of synthesis for diaminopurine derivatives and guanine derivatives is the same for a large number of steps. In order to prepare guanine derivatives instead of diaminopurine derivatives, a compound of formula II which could be used for diaminopurine synthesis, can be subjected to alkaline condition, thus replacing the substituent N in formula II by hydroxyl. This synthesis of guanine derivatives is also a subject of the present invention.

[0035] In another group of compounds of formula I, Z is defined to be an unprotected, partially protected or unprotected nucleic acid binding compound. The unprotected compounds can be used as nucleic acid binding compounds, for example as probes in assays for nucleic assays. Such assays are disclosed exemplary in WO 92/20703. Meanwhile, several improvements, especially for the synthesis of labelled PNAs and their use, where published. As outlined above, diaminopurine containing nucleic acid binding compounds have particular properties, especially in PNAs.

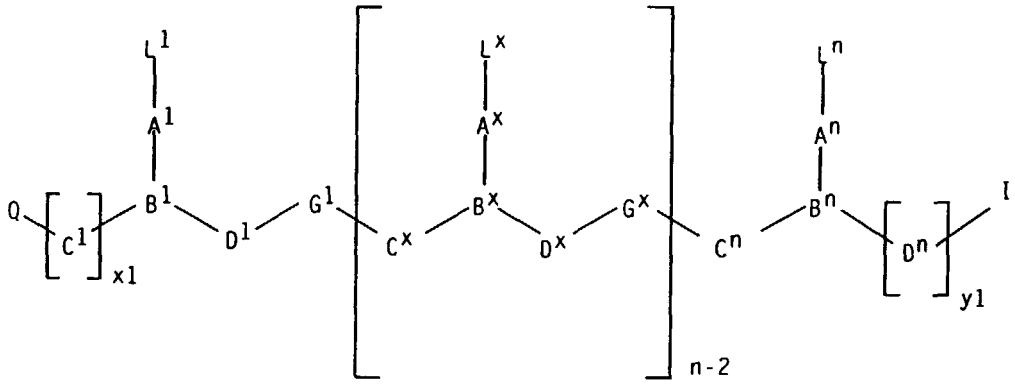
[0036] The controlled chemical synthesis of nucleic acid binding compounds usually comprises oligomerisation of monomeric backbone units which are suitably protected for avoiding side reactions, both of the monomer and of the already prepared oligomer, only letting free the functional groups for the intended coupling reaction. This new synthesis step can be used to prepare protected aminopurine moieties. It must be understood that protected nucleobases or their analogues, as aminopurine, are advantageously used during the synthesis of nucleic acid binding compounds because the extension of such nucleic acid binding compounds containing such protected diaminopurine moieties goes on without less side-reactions created by the amino groups if they were not protected. However, the prior art did not provide for easy synthesis of diprotected diaminopurine moieties.

[0037] The present invention, in contrast to the prior art, in a preferred embodiment will comprise the chemical synthesis of a precursor oligomer using at least one monomeric compound of formula II, wherein Z is a partially protected monomeric backbone unit. Such synthesis can be performed analogously to the method as for the monomers containing other (naturally occurring) nucleobases, avoiding alkaline conditions. The overall synthesis will yield an oligomer which is partially or totally protected, comprising at least one heterocyclic containing W. Thereafter, group W is replaced by T as outlined above, using nucleophilic replacement conditions.

[0038] If a nucleic acid binding compound contains more than one group wherein this replacement reaction is intended to occur, this may be done in one step. Further, under the conditions of this replacement reaction there may be further alterations within the nucleic acid binding compound or the monomeric backbone unit, such as simultaneous

removal of protecting groups. Surprisingly, this reaction can be conducted with high yield and again with a number of nucleophilic compounds of formula III. After oligomerisation and replacement, the partially or totally protected compound can be treated further, for example, any protecting group including those present in T, can be removed unselectively or selectively to attach reporter groups (like fluorescein), solid phases (like carboxyl group containing cellulose) or just to produce the binding properties of the oligomer.

[0039] Preferred molecules of formula I that can be prepared according to the present invention are compounds of the general formula VI



Formula VI

wherein

n is an integer of from at least 3,

x is an integer running from 2 to n-1,

each of L^1-L^n and L^x is a ligand independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L^1 -L^n and L^x s a diaminapurine modified as pointed out above and at least 2 of L^1-L^n are a naturally or non-naturally occurring nucleobase,

each of C^1-C^n and C^x is $(CR^6R^7)_y$ (preferably CR^6R^7, CHR^6CHR^7 or $CR^6R^7CH_2$) where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy, (C_1-C_6)alkoxy, or (C_1-C_6)alkylthio-substituted (C_1-C_6)alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system; or C^1-C^n is CO, CS, CNR^3;

each of D^1-D^n and D^x is $(CR^6R^7)_z$ (preferably CR^6R^7, CHR^6CHR^7 or $CH_2CR^6R^7$) where R^6 and R^7 are as defined above,

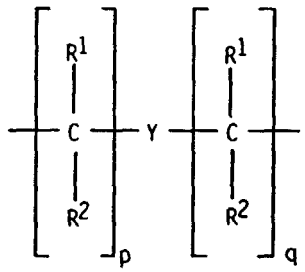
each of y and z is zero or an integer from 1 to 10, the sum y + z being at least 2, preferably greater than 2, but not more than 10,

each of G^1-G^{n-1} and G^x is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined below;

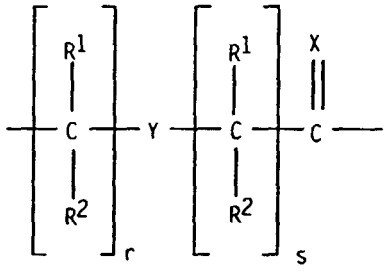
each of A^1-A^n and A^x and B^1-B^n and B^x are selected such that:

(a) A^1-A^n and A^x is selected from the group of formula (VII/A), (VII/B), (VII/C) or (VII/D), and B^1-B^n and B^x is selected from N or R^3N^+ or

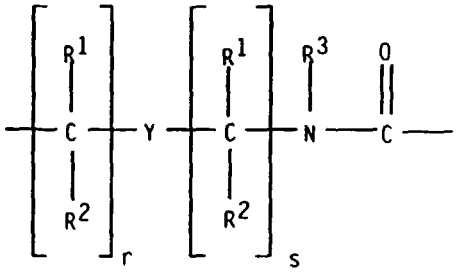
(b) A^1-A^n and A^x is selected from the group of formula (VII/D) and B^1-B^n and B^x is CH;



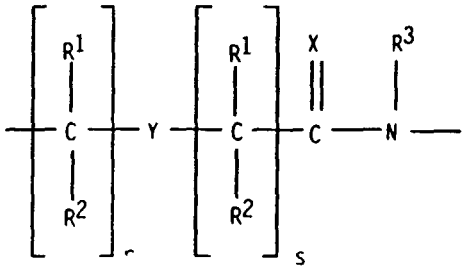
Formula VII/A



Formula VII/B



Formula VII/C



Formula VII/D

wherein:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4,

each of p and q is zero or an integer from 1 to 5, (the sum p+q being preferably not more than 5),

each of r and s is zero or an integer from 1 to 5, (the sum r+s being preferably not more than 5),

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or (C_1-C_4)alkoxy- or (C_1-C_4)alkylthio-substituted, hydroxy, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, amino and halogen,

each R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, (C_1 -C_6)-alkoxy, (C_1 -C_6)-alkylthio and amino,

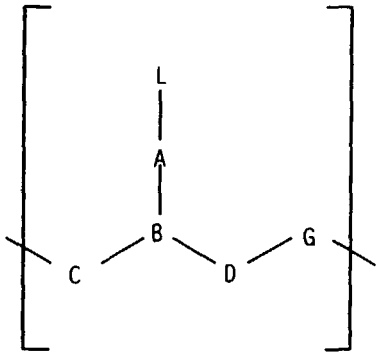
Q and I is independently selected from the group consisting of -CO_2H, -CONR'R'', -SO_3H and -SO_2NR'R'' ,an activated derivative of -CO_2H or -SO_3H and -NR'R''' and a solid phase bound derivative of -CO_2H, -CONR'R'', -SO_3H and -SO_2NR'R''.

where R', R'' and R''' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides,

nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides, soluble and non-soluble polymers, solid phases as well as nucleic acid binding moieties and

each of x1 and y1 is an integer of from 0 to 10.

[0040] Preferred compounds of formula I contain at least one monomer subunit of the formula VIII



Formula VIII

wherein for A, B, C, D, G, L the above definitions for A^x, B^x, C^x, D^x, G^x, and L^x apply.

[0041] Alkoxy- and alkylthio groups in formulae VI — VIII contain preferably from 1 to 4 carbon atoms.

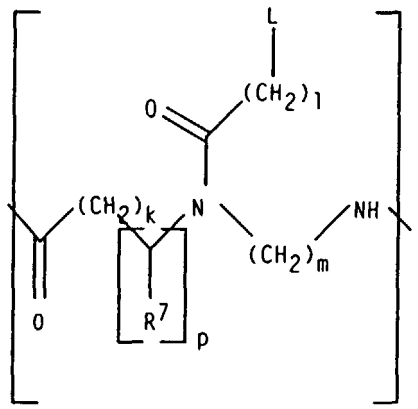
[0042] An example for a fused aromatic moiety is naphthol. A heterocyclic moiety is for example pyridin. Reporter groups are moieties that can be detected, like fluorescent compounds, for example fluorescein, or moieties that can be recognized by another molecular entity, like haptens which can be recognized by an antibody raised against this hapten.

[0043] In the above structures wherein Q or I is an oligonucleotide or oligonucleoside, such structures can be considered chimeric structures between PNA compounds and the oligonucleotide or oligonucleoside.

[0044] Linkers A^1-A^n for binding acceptor moieties are generally preferred at a length of between 1 to 10 atoms, most preferred 2 to 6 atoms, for donor moieties at a length of 1 to 10, most preferred 2 to 8 atoms.

[0045] Labels are generally known as moieties that are themselves immobilizable or detectable or can be immobilized/detected by coupling to additional moieties. Examples of labels are fluorescent moieties, (e.g. fluoresceine or rhodamine), enzymes, (e.g. peroxidase or phosphatase), immunologically active substances, like haptens, (e.g. digoxigenin), or protein binding tags (e.g. biotin) etc.

[0046] Most preferred nucleic acid binding compounds of formula I comprise at least one monomeric subunit of the general formula IX:



(IX)

wherein

L is a protected diaminopurine as pointed out above,
k, l and m is independently zero or an integer from 1 to 5,
p is zero or 1, and
R^7 is selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids.

Figure 1 is showing a flow-scheme of a first mode of synthesis of di-Z-protected diaminopurine.

Figure 2 is showing a flow-scheme of a synthesis of diisopropyl protected diaminopurine.

Figure 3 is showing a flow-scheme of a synthesis of diacetyl protected diaminopurine.

Figure 4 is showing a flow-scheme of a synthesis of mono-Z-protected diaminopurine

Figure 5 shows the synthesis of a protected diaminopurine containing PNA monomers 7, 8, 9, 11 and 12 as well as the (heterocyclus) unprotected monomer 10.

Figure 6 shows the nucleophilic displacement in a PNA oligomer, using as Nucleophile (:Nu) either ammonia or methyl amine.

Figure 7 shows the synthesis of a diisobutyryldiaminopurine monomer on an alternative route.

[0047] The following examples are explaining the invention in more detail.

Examples

General

[0048] All reagents used are analytical grade and from Aldrich. HATU was purchased from PerSeptive Biosystems (MA). MBHA resin were purchased from NovaBiochem (Switzerland).

Abbreviations

[0049] The following abbreviations are employed: triethylamine (TEA), *tert*-butyloxycarbonyl (Boc), dimethylformamide (DMF), dichloromethane (DCM), butyl (Bu), tetrahydrofurane (THF), dimethylsulfoxide (DMSO) and acetyl (Ac).

Example 1

Benzyl (2-amino-6-chloropurin-9-yl)acetate (2) Synthesis of Boc 6-Chloro-*N*^{2}-Isobutyryl Guanine Monomer (Figure 5)

[0050] A suspension of 2-amino-6-chloropurine (1) (25 g, 147 mmol) and potassium carbonate (41 g, 297 mmol) in DMF (500 ml) was stirred at room temperature for 1 h. Benzyl 2-bromoacetate (37 g, 25 ml, 162 mmol) was added in one portion and the reaction mixture was stirred for 3 h. After adding celite (10 g), the inorganic solid was removed by filtration through celite and the filtrate was evaporated to dryness in vacuo. The solid residue was suspended in DMF (125 ml) and cooled at 15°C, followed by filtration. The clear brown solution was evaporated in vacuo. The raw product was dissolved in hot ethanol (1.5 l) and kept over night at room temperature before filtration. This gave 34.6 g (74%) of the pure product 2. MS (+MALDI-TOF) 318.0 $[M+H]^+$; ^{1}H NMR (d6-DMSO) δ 8.13 (1H, s, H-8); 7.41-7.35 (5H, m, Ph); 7.00 (2H, s, NH_2); 5.21 (2H, s, CH_2Ph); 5.08 (2H, s, CH_2CO).

Benzyl (*N*^{2}-(Isobutyryl)-2-amino-6-chloropurin-9-yl)acetate (3)

[0051] To a solution of Benzyl (2-amino-6-chloropurin-9-yl)acetate (2) (1.0 g, 3.14 mmol) in pyridine (10 ml) at 0°C was added isobutyrylchloride (0.4 g, 3.75 mmol). The mixture was stirred at 0°C for 30 min, followed by 3 h at room temperature. The mixture was evaporated to dryness in vacuo and the residue was dissolved in hot ethyl acetate. The product precipitate slowly when standing at 0°C. Yield= 800 mg (66%). MS (+MALDI-TOF) 389.4 $[M+H]^+$; ^{1}H NMR (d6-DMSO) δ 10.82 (1H, s, NH); 8.52 (1H, s, H-8); 7.37 (5H, m, Ph); 5.23/5.22 (4H, 2xs, 2xCH_2); 2.82 (1H, m, CH-iBu); 1.10 (6H, d, CH_3-iBu).

(N^2-(Isobutyryl)-2-amino-6-chloropurin-9-yl)acetic acid (4)

[0052] Benzyl (N^2-(isobutyryl)-2-amino-6-chloropurin-9-yl)acetate (600 mg, 1.5 mmol) was dissolved in a mixture of H_2O (4 ml) and THF (4 ml) and 2 M NaOH (6 ml) was added. The mixture was left with stirring for 1 h. After adjusting the pH at 2.5 with 2M $NaHSO_4$ the mixture was extracted with ethyl acetate (3x100 ml). The organic phase was dried over $MgSO_4$ and the filtrate concentrated in vacuo. The resulting yellow oil was purified by column chromatography using DCM/MeOH/triethylamine (90:10:1) as eluent. This gave 360 mg (78%) of the title compound **4.** MS (+MALDI-TOF) 299.3 $[M+H]^+$; ^1H NMR (d6-DMSO): δ 10.76 (1H, s, NH); 8.42 (1H, s, H-8); 4.62 (2H, s, CH_2CO); 2.92 (6H, q, 3xCH_2-TEA); 2.80 (1H, m, CH-iBu); 1.10 (15H, m, 2xCH_3-iBu and 3xCH3-TEA).

Methyl *N*-((*N*^{2}-(Isobutyryl)-2-amino-6-chloropurin-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycinate (5)

[0053] Methyl *N*-(*tert*-Butyloxycarbonylaminoethyl)glycinate (260 mg, 1.12 mmol) was dissolved in DMF (5 ml) and (N^2-(Isobutyryl)-2-amino-6-chloropurin-9-yl)acetic acid (280 mg, 0.94 mmol), HOBt (160 mg, 1.2 mmol) and DCC (250 mg, 1.2 mmol) was added. After 2 h at room temperature the mixture was diluted with DCM and the solution was filtered and washed with 0.5 M $NaHCO_3$ and with 2 M $NaHSO_4$. After concentration in vacuo, the crude product was dissolved in ethanol/water and left over night at 4°C. The pure product was isolated upon filtration (320 mg, 0.62 mmol, 56%). MS (+MALDI-TOF) 512.0 $[M+H]^+$; ^1H NMR (d6-DMSO): δ 10.82 (1H, s, NH); 8.38 (1H, s, H-8); 7.07-6.74 (1H, m, NH); 5.28/5.11 (2H, 2xs, rotamers CH_2CO); 4.48/4.10 (2H, 2xs, rotamers CH_2CO); 3.62 (3H, s, OCH_3); 3.61-3.19 (4H, m, 2xCH_2); 2.84 (1H, m, CH-iBu); 1.32 (9H, s, 3xCH_3-tBu); 1.09 (6H, d, 2xCH_3-iBu).

***N*-((*N*^{2}-(Isobutyryl)-2-amino-6-chloropurin-9-yl)acetyl)-*N*-(2-Boc-aminoethyl) glycine (6)**

[0054] Methyl *N*-((*N*^{2}-(Isobutyryl)-2-amino-6-chloropurin-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycinate (220 mg, 0.43 mmol) was dissolved in a mixture of THF (1 ml) and water (1 ml). After addition of 1 M LiOH (1 ml, 1 mmol) the mixture was left at room temperature for 2 h. THF was removed *in vacuo* and the pH was adjusted to 2.5 with 2 M $NaHSO_4$. The product was obtained by extraction with ethyl acetate and concentration of the organic phase. This gave 155 mg (72%) of the title compound. MS (+MALDI-TOF) 498.4 $[M+H]^+$; ^1H NMR (d6-DMSO): δ 10.82 (1H, s, NH); 8.39 (1H, s, H-8); 7.07-6.73 (1H, m, NH); 5.27/5.11 (2H, 2xs, rotamers CH_2CO); 4.35/4.02 (2H, 2xs, rotamers CH_2CO); 3.54-3.03 (4H, m, 2xCH_2); 2.84 (1H, m, CH-iBu); 1.32 (9H, s, 3xCH_3-tBu); 1.08 (6H, d, 2xCH_3-iBu).

Example 2

Nucleophilic displacement in 6-chloropurin monomer

[0055] The final monomer 6 was dissolved in ammonia in water (32%), methylamine in water (40%) and piperidine in DMF (20%), respectively. The reaction was followed by HPLC and with MALDI-TOF MS. The substitution of 6-chloro takes place within minutes as seen by HPLC and MS. The removal of the isobutyryl group is more slowly, fastest in methylamine (complete in 5 h at room temperature) and very slow in piperidine (not complete in 72 hours). For definitions see figure 6

7: MS (+MALDI-TOF) 478.6 $[M+H]^+$
8: MS (+MALDI-TOF) 492.5 $[M+H]^+$
9: MS (+MALDI-TOF) 546.3 $[M+H]^+$
10: MS (+MALDI-TOF) 407.9 $[M+H]^+$
11: MS (+MALDI-TOF) 422.6 $[M+H]^+$
12: MS (+MALDI-TOF) 476.1 $[M+H]^+$

Example 3

Nucleophilic displacement of 6-chloropurin in PNA oligomer

[0056] The PNA Ac-TGT-ACG-TCG^{Cl}-CAA-CTA-Gly (**13**) was synthesized on an ABI 433A peptide synthesizer using Boc/Acyl monomers and *N*-((*N*^{2}-(Isobutyryl)-2-amino-6-chloropurin-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (**6**). The base-labile PAM linker to the MBHA-resin was introduced in the first cycle using Boc-Gly-PAM (double coupling). All cycles and reagents were standard as previously reported [Koch et al. J. Peptide Res. 49, 1997, 80-88]. The final PNA was split up in two tubes and treated in (A) ammonia in water (20 h at 60°C) and in (B) methylamine (5 h at room temperature).

14: MS (+MALDI-TOF) 4162.5 $[M+H]^+$

15: MS (+MALDI-TOF) 4180.0 $[M+H]^+$

Example 4

Synthesis of *N*-((*N^2,N^6*-(diisobutyryl)-2,6-diaminopurine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (21) (Figure 8)

Ethyl (2,6-diaminopurin-9-yl)acetate **(17)**

[0057] To a suspension of 2,6-diaminopurine (16) (15 g, 100 mmol) in DMF (25 ml) was added K_2CO_3 (41 g, 300 mmol) and the mixture was stirred for 1 h at room temperature. After addition of ethyl bromoacetate (13.3 ml, 120 mmol) the suspension was stirred for additional 3 h. The reaction mixture was filtered and the residue was washed with DMF (100 ml). The filtercake was boiled with abs. EtOH (200 ml) and filtrated. The filtrate was boiled with activated carbon and filtered through 5 mm celite. The volume of the filtrate was reduced to 30 ml and cooled whereby the compound **17** precipitated (12.5 g, 53 mmol) (53 %).

^{1}H NMR (d6-DMSO) δ 7.69 (1 H, 8-H); 6.72 (2 H, NH_2); 5.83 (2 H, NH_2); 4.85 (2 H, CH_2CO); 4.15 (2 H, OCH_2); 1.2(3 H, CH_2CH_3).

Ethyl ((*N^2,N^6*-diisobutyryl)-2,6-diaminopurine-9-yl)acetate (18)

[0058] Isobutyrylchloride (2.9 ml, 27.4 mmol) was added to a suspension of ethyl (2,6-diaminopurin-2-yl)acetate (1.6 g, 6.8 mmol) in pyridine (20 ml) at 0°C. The mixture was stirred for 2 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was taken up with DCM and extracted with diluted HCl. The organic phase was dried with $MgSO_4$ and evaporated to dryness in vacuo. The residue was crystallized from abs. EtOH to give 2.35 g of the title compound 18 (92%).

MS (+MALDI-TOF) 377 $[M+H]^+$; ^{1}H NMR (d6-DMSO) δ 10.48 and 10.99(2 H, NH); 8.29(1 H, 8-H); 5.09(2 H, CH_2CO); 4.18 (2 H, OCH_2); 3.06 and 2.93 (2 H, 2xCH-iBu); 1.22(3 H, $CH_2C\underline{H}_3$); 1.10(12 H, 4xCH_3-iBu).

((*N^2,N^6*-diisobutyryl)-2,6-diaminopurine-9-yl)acetic acid (19)

[0059] Compound 18 (2.2 g, 5.85 mmol) was stirred for 2 h in a 2:1 mixture of water /THF (50 ml). The compound was completely dissolved after addition of 1 M LiOH (16 ml). The pH of the solution was adjusted to 2.5 with 2 M $NaHSO_4$ and extracted with DCM (2x50 ml). The organic phase was extracted with brine, dried with $MgSO_4$ and evaporated to dryness *in vacuo.* Crystallization from abs. Ethanol gave 1.9 g (95%) of ((N^2,N^6-diisobutyryl)-2,6-diaminopurine-2-yl)acetic acid.

MS (+MALDI-TOF) 349 $[M+H]^+$.

Methyl *N*-((*N^2,N^6*-(diisobutyryl)-2,6-diaminopurine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (20)

[0060] ((N^2,N^6-diisobutyryl)-2,6-diaminopurine-9-yl)acetic acid (1.5 g, 4.4 mmol), DCC (1 g, 1.1 eq.), HOBt (0.66 g, 1.1 eq.) and methyl N-(Boc-aminoethyl)glycinate (1.23 g, 1.2 eq.) was stirred in DMF (30 ml) over night. The reaction mixture was filtrated and the filter was washed with DCM (2x30 ml). The filtrate was extracted with diluted $NaHCO_3$ (3 x 20 ml), 2 M $NaHSO_4$ (2 x 20 ml) and with brine (30 ml). The organic phase was dried with $MgSO_4$ and evaporated to dryness *in vacuo.* Crystallization from ethanol gave 2.2 g (90 %) of methyl N-((N^2,N^6-(diisobutyryl)-2,6-diaminopurine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycinate. MS (+MALDI-TOF) 562 $[M+H]^+$.

***N*-((N^2,N^6-(diisobutyryl)-2,6-diaminopurine)-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (21)**

[0061] Methyl N-((N^2,N^6-(diisobutyryl)-2,6-diaminopurine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine was used without further purification (2.0 g, 3.6 mmol) and was stirred in a 10:1 mixture of water and THF (40 ml). 1 M LiOH (6 ml) was added and the compound 20 was completely dissolved. The solution was adjusted to pH 2.8 with 2 M $NaHSO_4$ and extracted with DCM (2x30 ml). The organic phase was dried with $MgSO_4$ and evaporated to dryness *in vacuo.* Crystallization from ethanol gave 1.8 g of the title compound (92%). MS (+MALDI-TOF) 548 $[M+H]^+$; ^{1}H NMR (d6-DMSO) δ 10.51 (s, 1 H, NH); 10.32 (s, 1 H, NH); 8.16 and 8.15 (s, 1 H, 8-H); 7.04 and 6.76 (m, 1 H, BocHN); 5.21 and 5.10 (s, 2

H, CH_2CO); 4.35 and 4.00 (s, 2 H, CH_2CO); 3.5-2.8 (m, 6 H, 2xCH_2 and 2xCH-iBu); 1.35 and 1.33 (s, 9 H, CH_3-tBu); 1.1 (m, 12 H, CH_3-iBu).

Example 5

Preparation of di-benzyloxycarbonyl-protected diaminopurine PNA-monomer Benzyl (N2-(benzyloxycarbonyl)-2-amino-6-chloropurin-9-yl)acetate (22)

[0062] A mixture of 33 g (105 mmol) benzyl (2-amino-6-chloropurin-9-yl)acetate (2), 1 l tetrahydrofuran and 23 g (231 mmol) triethylamine was stirred in a nitrogen atmosphere and cooled to 0°C. A solution of 12.5 g (42 mmol) triphosgene in 200 ml of tetrahydrofuran was added to the suspension in one portion and the reaction mixture was stirred for 2 hours at 0 °C. Benzyl alcohol 13.6 g (126 mmol) was added in one portion and the reaction mixture was stirred for another hour. Triethylamine was removed by filtration and washed with 3x25 ml of tetrahydrofuran and the filtrate was evaporated to dryness. The residue was refluxed in 300 ml ethanol and filtered. After cooling to 10 °C the target compound was isolated by filtration and washed with 2x25 ml ethanol. The product was dried in vacuum and yielded 35.7 g of benzyl (N2-(benzyloxycarbonyl)-2-amino-6-chloropurin-9-yl)acetate.

mp. 153-155°C

^{1}H NMR (DMSO,d_6),δ(ppm): 5.2-5.22 (m, 6H), 7.33-7.47 (m, 10H), 8.51 (s, 1H),10.83 (s, 1H, NH).

Anal. cald. for $C_{22}H_{18}Cl\ N_5O_4$: C, 58.48; H,4.02; Cl, 7.85; N,15.50. Found: C, 58.31; H,4.05; ^{13}C NMR (DMSO,d_6),δ(ppm): 44.51, 65.98, 66.97, 126.80, 127.76, 128.00, 128.09, 128.34, 135.30, 136.57, 146.76, 149.26, 151.80, 152.44, 153.16, 167.36.

C17.94; N, 15.63.

(N^2,N^6-di(benzyloxycarbonyl)-2,6-diamino-purine-9-yl)acetic acid (23)

[0063] A solution of 7 g (46 mmol) of benzylcarbamate in 100 ml of dry THF (dried over aluminium oxide) was added 1.9 g (47,5 mmol) of sodium hydride (60 % dispersed in oil) and stirred for 1 hour at room temperature. The white slurry was added 10 g (22.2 mmol) of benzyl (N2-(benzyloxycarbonyl)-2-amino-6-chloropurine-9-yl)acetate the solution was stirred 4 h at room temperature. The reaction mixture was poured in 400 ml of cold abs. ethanol and stirred for 10 min.. The precipitate was collected and crystallized from abs. ethanol and gave 6.2 g 59 % of the target compound.

^{1}H NMR (DMSO,d_6),δ(ppm): 11.34 (s,1H, NH), 10.78 (s, 1H, NH), 8.41 (s, 1H, H-8), 7.34-7.44 (m, 10H, aromates), 5.32 (s, 1H, CH_2COO), 5.23 (s, 1H, CH_2benzyl), 5.18 (s, 1H, CH_2benzyl).

Methyl *N*-((*N^2,N^6*-di(benzyloxycarbonyl)-2,6-diamino-purine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycinate(24)

[0064] A solution of 1 g (2.1 mmol) (N^2,N^6-di(benzyloxycarbonyl)-2,6-diamino-purine-9-yl)acetic acid in 25 ml of dry DMF was stirred at room temperature and added 0.8 g (2.1 mmol) HATU and 0.57 g (2.1 eqv.) of DIEA. The solution was stirred for additional 15 min. and 1g (4.3 mmol, 2.05 eqv.) of methyl *N*(2-*tert*--butoxycarbonylaminoethyl)glycinate was added the temperature was raised and solution was stirred over night at 60°C. The solution was cooled to room temperature and added 50 ml of DCM and extracted with 3x25 ml diluted sodium hydrogen carbonate, 2x25 ml sodium hydrogen sulfate and brine. The organic phase was dried over magnesium sulfate, filtered and the volume was reduced to 7 ml and applied to a silica 60 column (40 g, d=30 mm) where it was eluted with 0-2 % methanol in DCM. The pure fractions was collected and evaporated to dryness giving 352 g (0,51 mmol, 24 %) of the target compound.

Example 6

Alternative synthesis of 24

[0065] A solution of 403 mg (0.85 mmol) (N^2,N^6-di(benzyloxycarbonyl)-2,6-diamino-purine-9-yl)acetic acid (23) in 5 ml DCM/DMF 3:2 was added 175 mg (0.853 mmol) DCC and 115 (0.85 mmol) HOBT. The solution was stirred for two days after which more than 80 % of the acid was activated (HPLC). The solvent was removed under reduced pressure. The residue was dissolved in 5 ml of DMF and added 592 mg (3 eq.) of methyl *N*(2-*tert*--butoxycarbonylaminoethyl)glycinate. The reaction was stirred for additional 13.5 days where it reached a plateau of 82 % (HPLC) converted to methyl

N-(*N*^{2},*N*^{6}-di(benzyloxycarbonyl)-2,6-diamino-purine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycinate. The reaction mixture was filtered and added 20 ml of DCM and extracted with 3 X $NaHCO_3$, 2 X $NaHSO_4$ and brine. The organic phase was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. Crystallization from MeOH/ Water 1:1 gave 361 mg of the target compound work up of the mother liquor gave additional 63 mg of the target compound bringing the yield to 73 %.

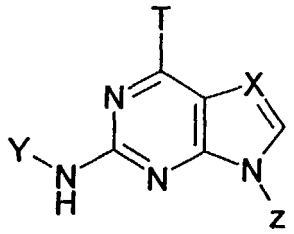
Example 7

Synthesis of a benzyloxycarbonyl-protected diaminopurine PNA monomer (25) *N*-((*N*^{2},*N*^{6}-di(benzyloxycarbonyl)-2,6-diamino-purine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (25)

[0066] A solution of 320 mg of Methyl *N*-((*N*^{2},*N*^{6}-di(benzyloxycarbonyl)-2,6-diamino-purine-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycinate (4) in 14 ml THF was cooled to 0°C and added 4 ml of water, 0.520 ml (1.1 eq.) 1 M KOH and 0.1 ml (0.2 eq.) 1 M KCN. The cooling was removed and the solution was stirred over night at room temperature. The solution was added 0.1 ml (0.1 eq.) 1 M KOH and stirred for additional tow hours. The reaction was added 10 ml of water and the pH was adjusted to 2.8 and then extracted with DCM. The organic phase was dried with brine and $MgSO_4$ and evaporated to dryness. Crystallization from EtOH gave 25.

Claims

1. Subject of the invention is therefore a method for the synthesis of compounds of the general formula I



(I)

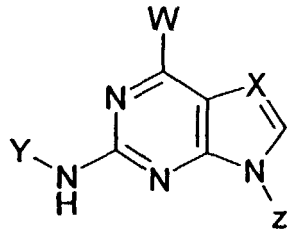
wherein

Ti is NV^1V^2

wherein

V^1 and V^2 are linked together other than via the N shown in the definition by a chain of at least 5 atoms, or is NHV, wherein V is selected from the group consisting of alkyl, a solid phase, a protecting group, and a reporter group
X is CH or N,
Y is an electron withdrawing protecting group, preferably a group belonging to the group of acyl groups, unsubstituted or substituted by, and
Z is a not electron withdrawing group, preferably is an electron donating group, most preferably selected from the group consisting of hydrogen (H), alkyl, unsubstituted or sustituted by a substituent selected from the group of SO_3H, PO_4H, COOH, CN and derivatives, like esters, thioesters or amides, thereof, said derivatives including, but being not restricted to an unprotected or protected nucleic acid binding compound, a partially or fully protected monomeric backbone unit of a nucleic acid binding compound,

characterized in that a compound of general formula II



wherein
the definitions of X, Y and Z are chosen from the possible definitions of X, Y and Z in formula I and

W is an electron withdrawing group, preferably selected from the group consisting of NO_2, halogen, Tos and Mes,

is reacted with a compound of general formula III,

T-H (III)

wherein

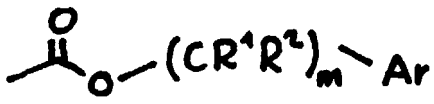
T is defined as in formula I,

under conditions favoring the elimination of a compound of the formula WH.

2. The method of claim 1, wherein Z is the group $-(CR^1R^2)_n - CO - OR^3$ wherein

R^1 and R^2 for each C at different n is chosen independently from the group of hydrogen, alkyl, aryl or allyl or R^1 and R^2 at a C at a defined position are together oxygen and
R^3 is a carboxylic acid protecting group and n is an integer of 1 - 5.

3. The method of claim 1, wherein V is the group

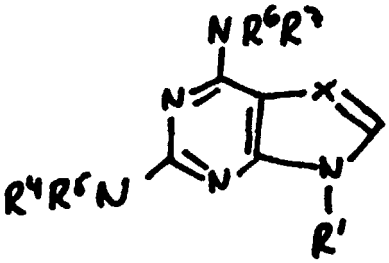


wherein

R^1 and R^2 chosen independently from the group consisting of hydrogen, alkyl, aryl or allyl or R^1 and R^2 at one C at a defined position are together oxygen
m is an integer of from 0 to 10, preferably from 1 to 3, and
Ar is an aromatic group of less than 15 carbon atoms, preferably of from 6 to 10 carbon atoms.

4. The method of claim 2, wherein under said conditions also group R_3 is replaced by hydrogen or a metal ion or an ammonium ion.

5. A compound of general formula IV



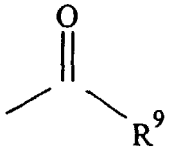
wherein

R' is the electron donating group $(CR^1R^2)_n$ $COOR^3$
R^4, R^5, R^6, R^7 are independently selected from the group consisting of H and R^8 wherein at least one of R^6 or R^7 is not H,
R^3 is -H, alkyl (unsubstituted or unsubstituted as above), a monomeric backbone unit or a totally or partially protected or unprotected nucleic acid binding compound,
R^8 is alkyl or a protecting group, preferably selected from the group consisting of acyl, aralkyl, carbonyloxyalkyl and carbonyloxyaralkyl,
n is an integer of between 1 and 5,
X is N or CH.

6. A method according to claim 1 wherein Z is a partially or totally protected not naturally occurring nucleic acid binding compound.

7. A compound of claim 6, wherein at least one of R^4 or R^5 is not H or alkyl.

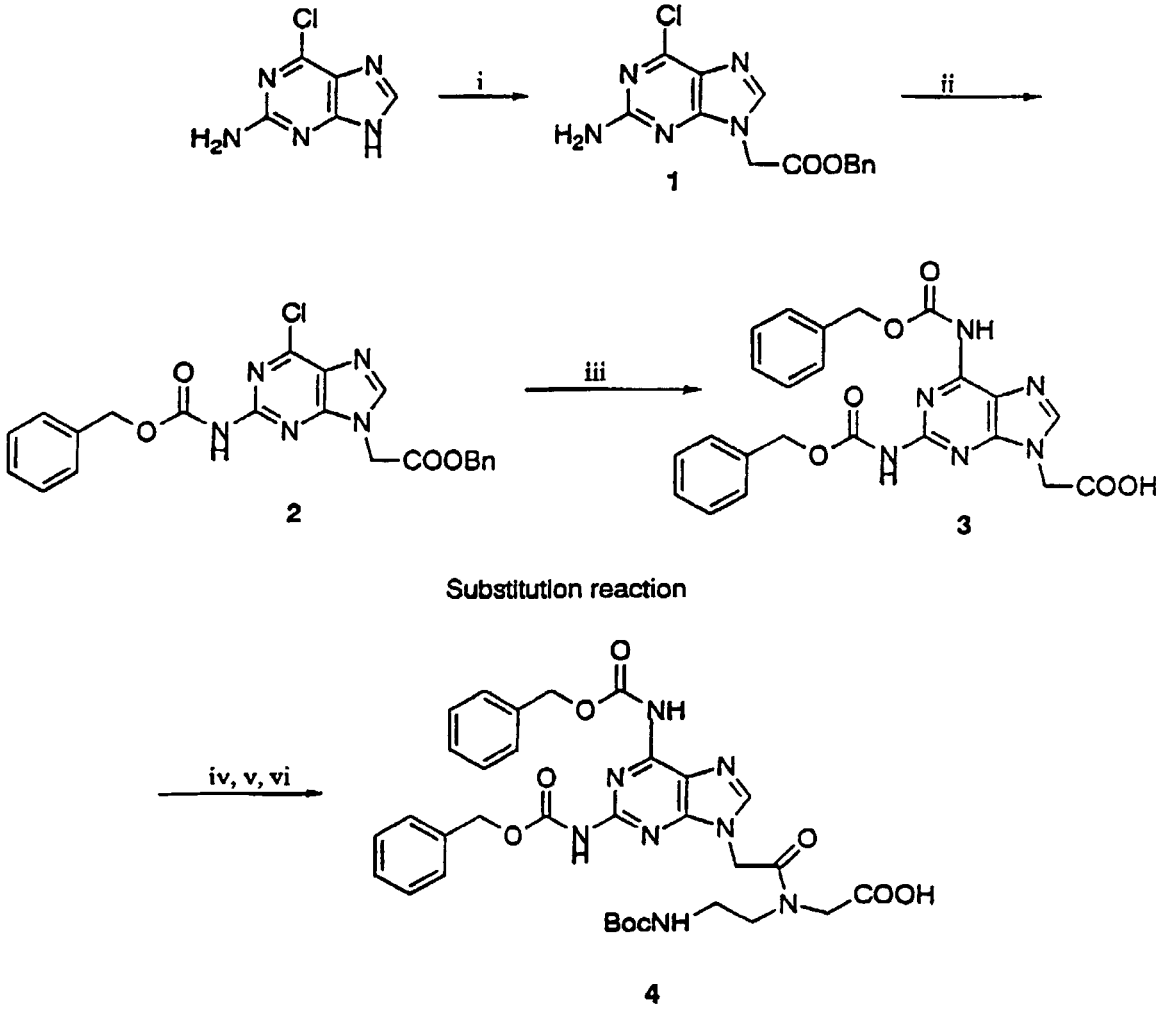
8. A compound of claim 6, wherein one of R^6 or R^7 and one of R^4 or R^5 is



wherein

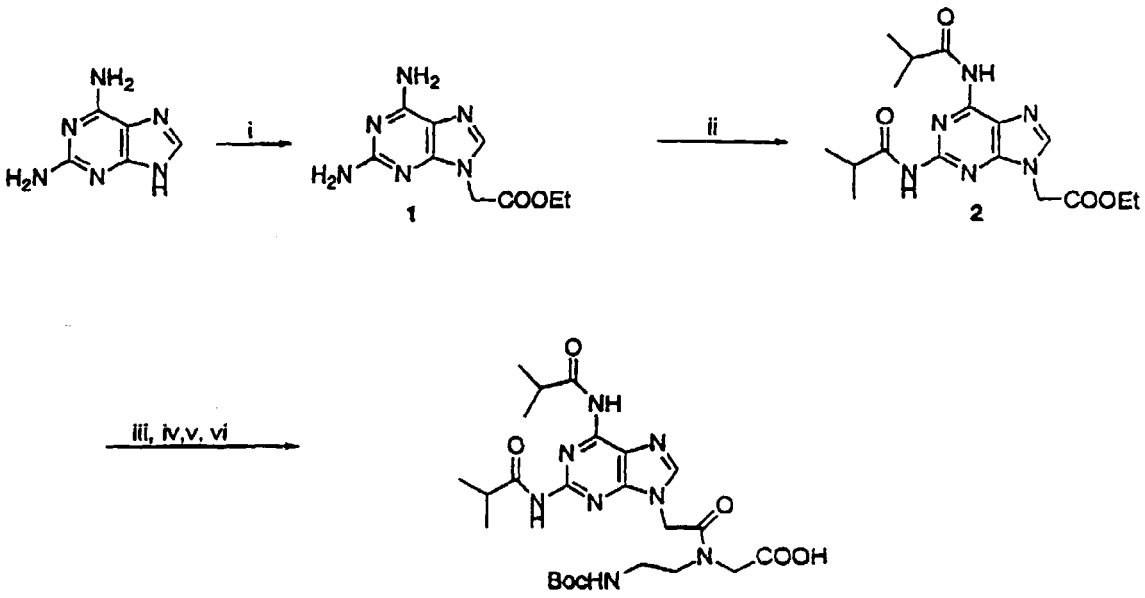
R^9 is selected from the group of substituted or unsubstituted alkyl, aryl, aralkyl and OR^{10},
wherein R^{10} is H, alkyl, allyl, a reporter group or aryl.

FIG 1



I: benzylbromoacetate, K_2CO_3,DMF. ii: $(CCl_2)CO$, benzylalkohol, TEA, THF.
iii : NaH, benzyl carbamate, THF. iv: DCC, HOBT, methyl *N*-(2-BOC-aminoethyl) glycinate, DMF
v: 1 N LiOH, THF, H_2O. vi: 2 N Na_2HSO_4.

FIG 2



i: Ethylbromoacetate, K_2CO_3,DMF. ii: $(CH_3)_2CHCOCl$, pyridine.
iii :2.5 eq LiOH, H2O. iv: DCC, HOBT, methyl *N*-(2-BOC-aminoethyl) glycinate, DMF.
v: 1 N LiOH, THF, H_2O. vi: 2 N Na_2HSO_4.

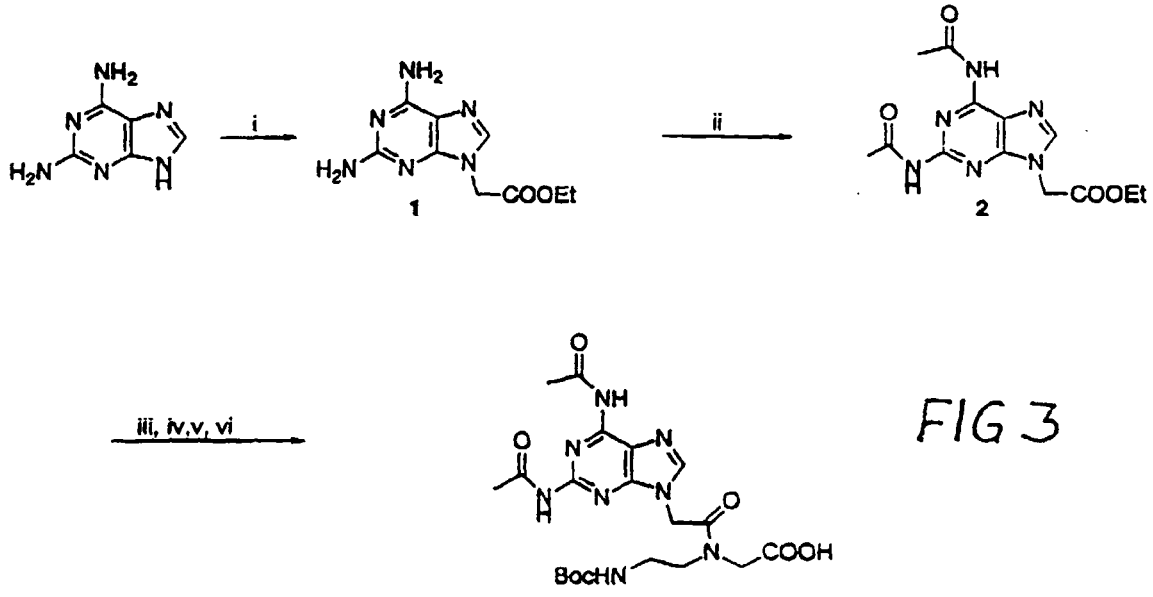
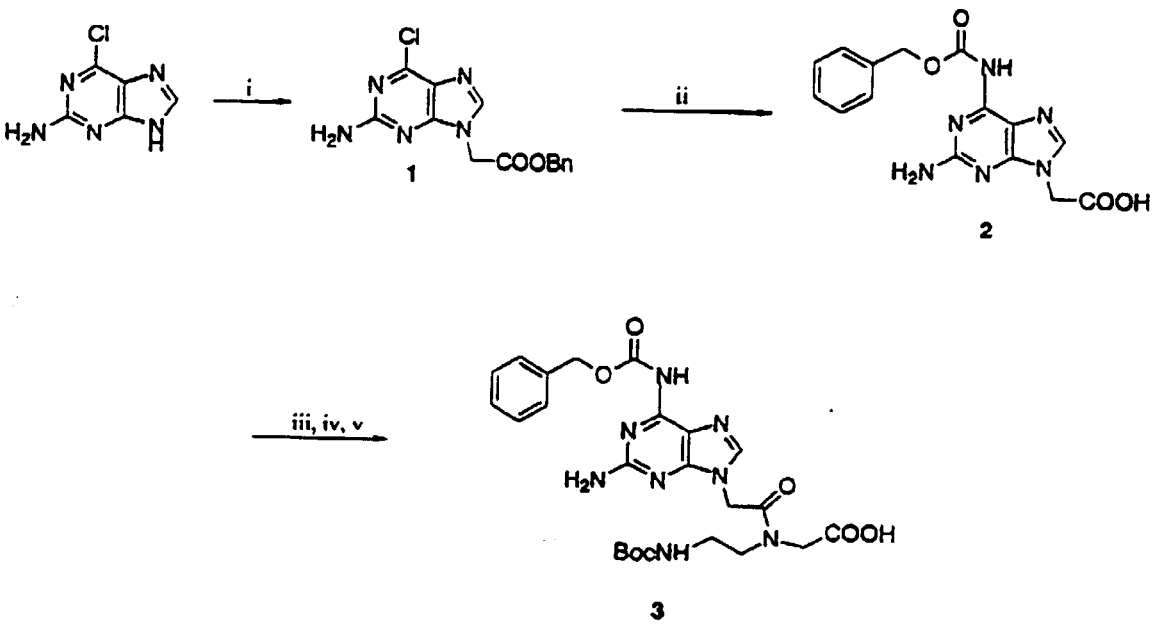


FIG 3

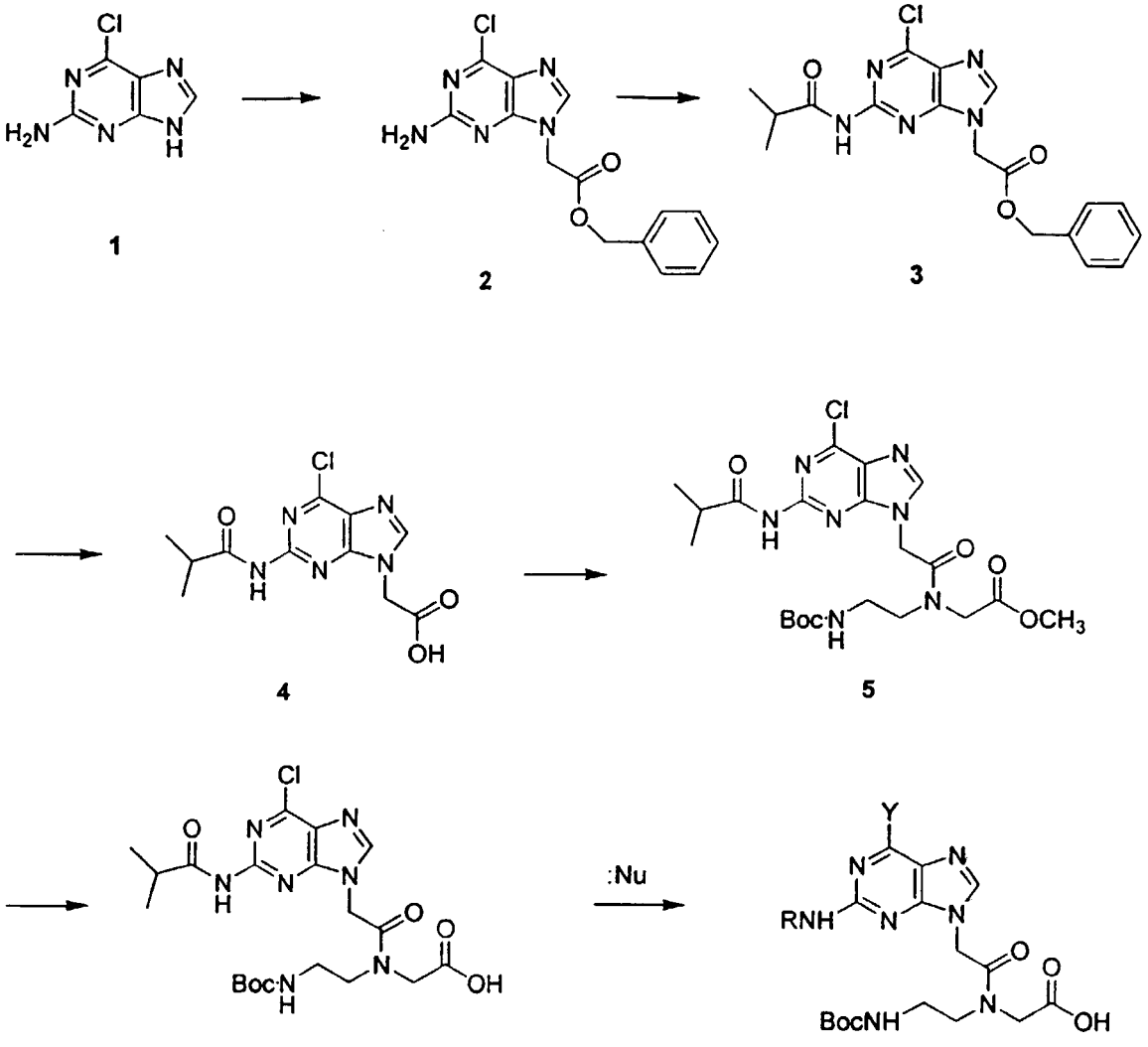
i: Ethylbromoacetate, K_2CO_3,DMF. ii: CH_3COCl, pyridine.
iii :2.5 eq LiOH, H2O. iv: DCC, HOBT, methyl *N*-(2-BOC-aminoethyl) glycinate, DMF.
v: 1 N LiOH, THF, H_2O. vi: 2 N Na_2HSO_4.

FIG 4



i: benzylbromoacetate, K_2CO_3,DMF. ii : NaH, benzyl carbamate, THF. iii: DCC, HOBT, methyl *N*-(2-BOC-aminoethyl) glycinate, DMF. iv: 1 N LiOH, THF, H_2O. vi: 2 N Na_2HSO_4.

FIG 5



7: R=IsoButyryl and Y=NH_2
8: R=Isobutyryl and Y=NHMe
9: R=Isobutyryl and Y=piperidine
10: R=H and Y=NH_2
11: R=H and Y=NHMe
12: R=H and Y=piperidine

FIG 6

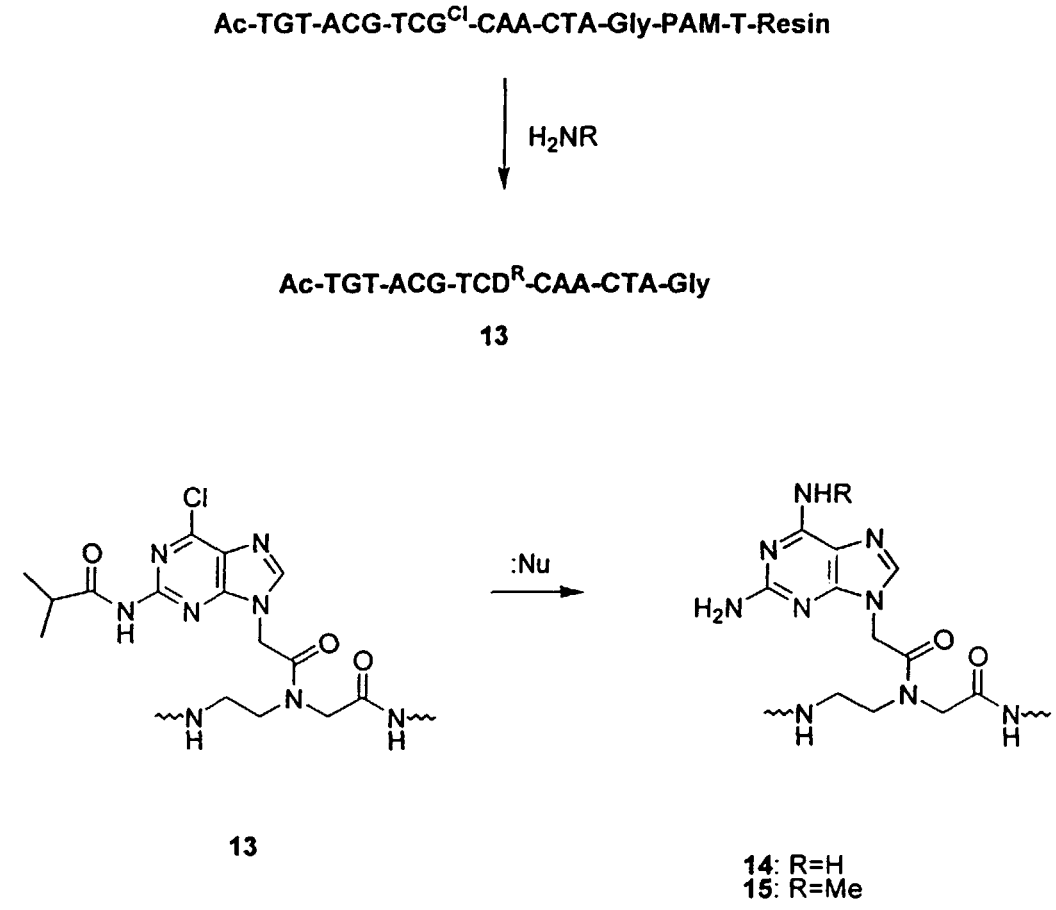
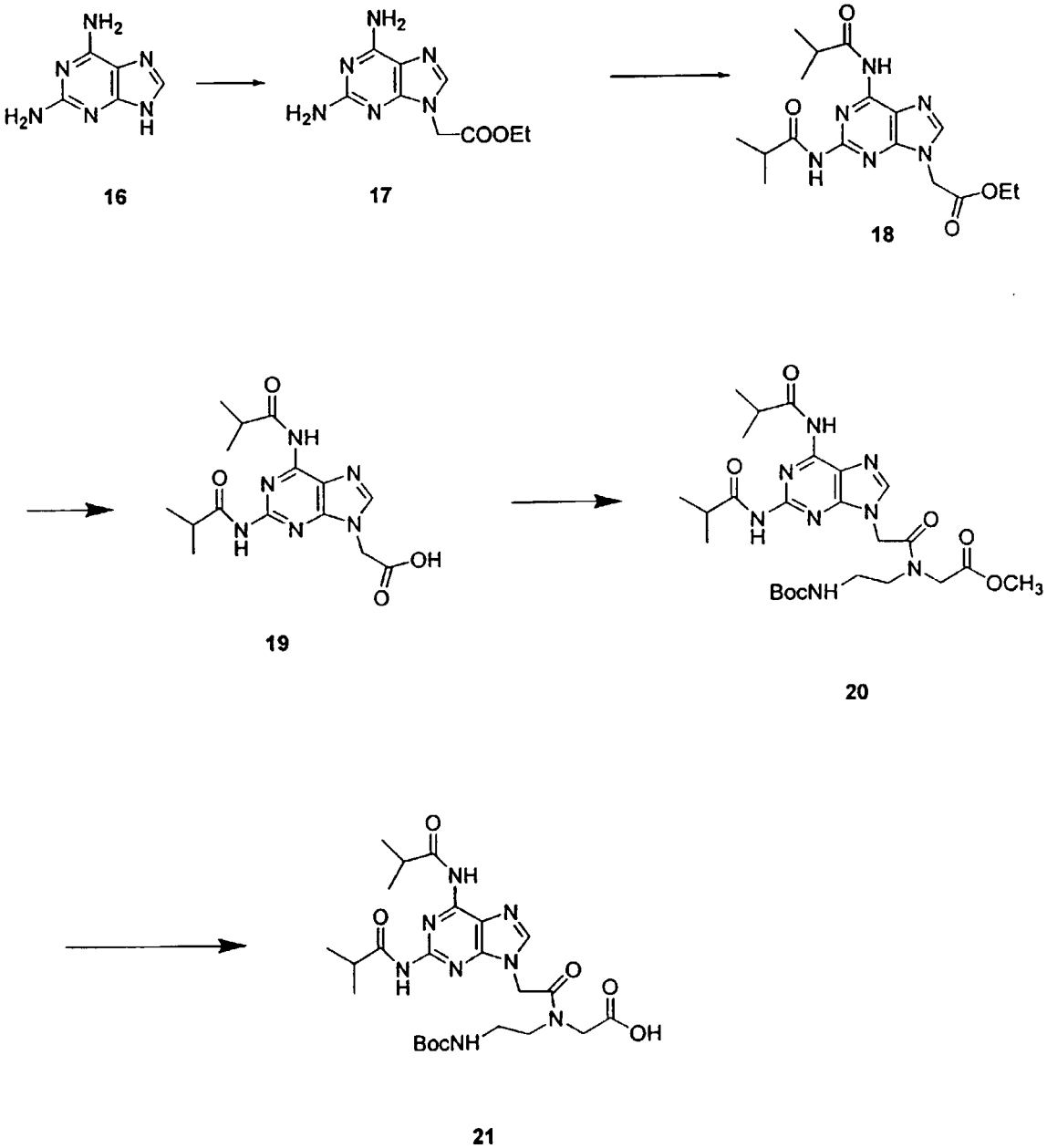

Ac-TGT-ACG-TCG^{Cl}-CAA-CTA-Gly-PAM-T-Resin
H_2NR
Ac-TGT-ACG-TCD^{R}-CAA-CTA-Gly
13
Cl
:Nu
NHR
H_2N
13
14: R=H
15: R=Me

FIG 7



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EUROPEAN SEARCH REPORT

Application Number
EP 00 20 3172

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	EP 0 212 535 A (BOEHRINGER MANNHEIM GMBH) 4 March 1987 (1987-03-04) * claims; examples 13,14,18,19 * ---	1-8	C07D473/16 C07D487/04 //(C07D487/04, 239:00,209:00)
Y	WO 99 07705 A (MEIJER LAURENT ;SCHULTZ PETER (US); KIM SUNG HOU (US); UNIV CALIFO) 18 February 1999 (1999-02-18) * claims; examples * ---	1-8	
Y	WO 98 16528 A (CHIRON CORP ;UNIV CALIFORNIA (US)) 23 April 1998 (1998-04-23) * page 6; claims * ---	1-8	
P,Y	WO 00 43394 A (HANU & SCARON ;HAJDUCH MARIAN (CZ); KRY & SCARON (CZ); LACHEMA AS) 27 July 2000 (2000-07-27) * claims * -----	1-8	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7) C07D

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	9 January 2001	Chouly, J

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09-01-2001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0212535 A	04-03-1987	DE 3529497 A	26-02-1987
		AT 63914 T	15-06-1991
		DE 3679474 D	04-07-1991
		JP 1963264 C	25-08-1995
		JP 6092407 B	16-11-1994
		JP 62045588 A	27-02-1987
		US 4853386 A	01-08-1989
WO 9907705 A	18-02-1999	AU 8773098 A	01-03-1999
		EP 1003746 A	31-05-2000
WO 9816528 A	23-04-1998	AU 4920397 A	11-05-1998
		US 6153618 A	28-11-2000
WO 0043394 A	27-07-2000	AU 2276100 A	07-08-2000

EPO FORM P0459

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